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Reproductive and Developmental Toxicity of the Components of Gasoline

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The reproductive, developmental, and postnatal toxicity of 14 select chemicals and mixtures that are components of gasoline has been reviewed. The majority of experimental analyses have been performed as either variations of the accepted segment 2 protocol or as traditional teratology studies. Specific deficiencies in the present database have been identified and are most obvious in the evaluation of reproductive and postnatal effects. It is recommended that future studies address the continuing need for assessment in multiple species and over a range of dosages with specific emphasis on the impact of route of administration on the results obtained.

Introduction

In all mammalian species, including the human, the normal completion of the reproductive process requires that several interdependent biological processes occur in sequence and that they occur properly. These events are *a*) germ cell production and release in the male parent; *b*) germ cell production and release in the female parent; *c*) gamete fusion and subsequent fertilization of the secondary oocyte; *d*) implantation of the blastocyst into the hormonally primed uterus; *e*) development and growth of the conceptus through embryogenesis and fetal development and maturation, and *f*) a normal delivery (*1–5*). The ultimate success of this sequence requires a precise interaction and balance among several hormones, the genetically programmed changes in morphology that occur during each phase, and the subsequent integration of several physiological events.

In the context of modern toxicology, each of these biological processes, which are under genetic and hormonal control, is a potential target for xenobiotics. Of major and overriding concern in any evaluation of reproductive and developmental toxicity is the realization that integration of these events is absolutely dependent on when they occur in time (*6,7*). It is an important corollary that any alteration or deviation from this precise program, at any stage, has the potential to result in an adverse outcome of pregnancy. It is of additional significance to recognize that chemical (xenobiotic) exposure at one time in this reproductive program can show an effect at a later time. Thus, exposure of the embryo to a chemical could alter the development of discrete cell populations (target cells) and result in morphological, biochemical, or physiological changes occurring after birth (*8*). These changes may not only be structural alterations that are commonly referred to as birth defects, but may also be functional changes such as the impaired development of specific nerve cells leading to mental retardation (*7–9*).

Over the past 25 years, efforts have been made to incorporate this unique and complex biology into definitive testing protocols that involve the traditional test animal species: rats, mice, and rabbits. These protocols have been designed to encompass all phases of the complete reproductive sequence, although the specific details of these tests are known to vary from country to country and from agency to agency (*10–12*). However, the specific evolution of these protocols focused on the development of three distinct and time-dependent tests. As diagrammed in Figure 1 (*13*), they are designated as segments of the reproductive process. In the segment 1 study, male and female rodents are treated separately over a time span designed to encompass the time required to complete an entire spermatogenic cycle (males) or four estrous cycles (females). Treated animals are then mated, and the females continue to be treated throughout the periods of gestation and lactation. The judicious use of this protocol permits the assessment of a broad spectrum of reproductive and developmental end points including gonadal function, embryogenesis, and the growth and viability of newborn pups.

The segment 2 study involves the exposure of pregnant females throughout the embryogenesis period of pregnancy and, traditionally, has been designated as the teratology study. Pregnant females are exposed (day 6–day 15) from the time immediately after the implantation process through the time when all organ systems can be recognized. This time period is commonly referred to as the phase of major organogenesis (*14*). Although this test

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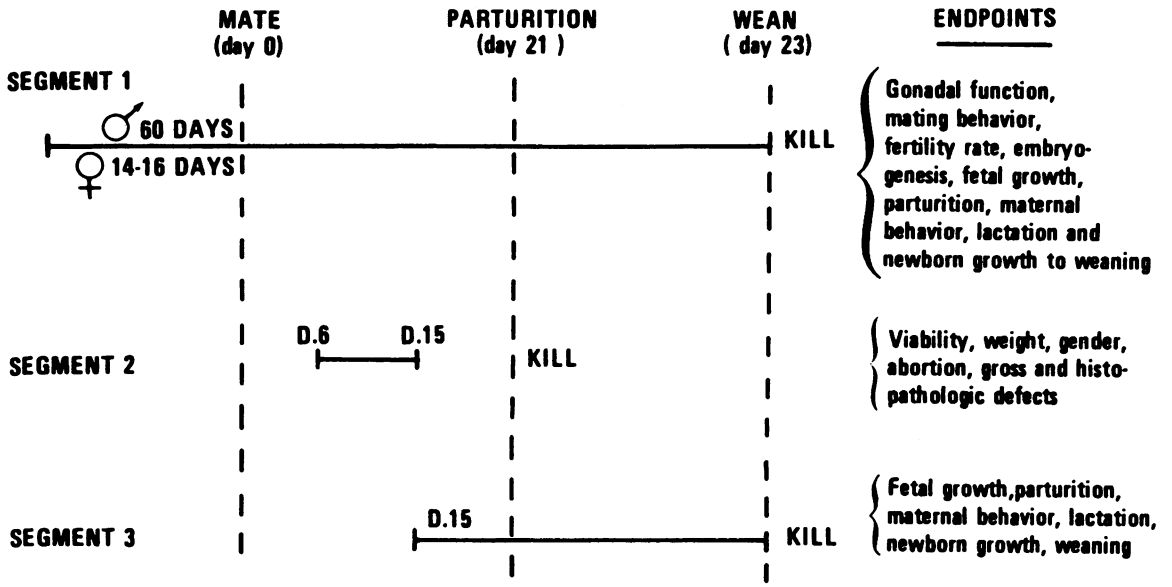

FIGURE 1. The generalized protocol used for developmental and reproductive toxicity studies in rodents. Each of the segments overlap in time; they encompass the entire spectrum of reproduction and development. Reprinted with permission (*13*).

is designed to evaluate the occurrence of birth defects per se as the outcome of maternal exposure, it also provides data on fetal viability and fetal weight.

When the segment 3 study is used, the pregnant females are exposed from late pregnancy (the fetal period of intrauterine development) through the periods of parturition and lactation. A judicious use of this testing protocol allows an investigator to monitor, among other things, postnatal growth and lactation. This study can also be extended and modified to permit the evaluation of postnatal behavioral effects of exposure (*12*), a test end point that can also be attained by modification of the basic segment 1 protocol. It is important to emphasize that each of these tests overlap in time so that some processes can be evaluated (e.g., lactation) under different test regimens. What is not indicated in Figure 1 are two important aspects of each of these tests that are absolutely critical for a meaningful assessment. First, for a complete analysis of the effects observed, the use of at least two species is required (*8*) and at least three doses should be used (*12*). With respect to potential human risk resulting from exposure to the same chemical, it is of equal importance that experimental design use the route of exposure most likely to occur in humans. As the companion disciplines of reproductive and developmental toxicology continue their scientific evolution and expand their database, it is becoming clear that the route of administration is of prime importance in determining the level of a test chemical, or an active metabolite, that reaches target cell populations in either the gonads (*15*) or the maternal-embryo unit (*16*) at susceptible periods of development.

In consort with the several reports included in this symposium, the working group that analyzed the issue of reproductive and developmental effects of the components of gasoline in the traditional test animal species relied primarily on literature citations obtained through Medline and Toxline. These were coupled with citations obtained independently, and this communication represents an appropriate review of the literature that was available through 1991. No claim is made for exhaustivity because certain restrictions were placed on the data used, particularly with respect to the use of published abstracts and the literature from Eastern and Central Europe that was not available in translation. Most of this information is available, however, and has been reviewed in earlier publications (*17–19*). In response to the charge given to our working group, this report represents an analysis of 14 materials found in gasoline or its exhaust or that are closely related to gasoline. These compounds were gasoline itself and related streams (high-flash aromatic naphtha, high aromatic solvent, rubber solvent, and Stoddard solvent) and several additional components and additives (ethanol, methanol, toluene, benzene, formaldehyde, xylene, 1,30-butadiene, methyl tertiary butyl ether, and hexane). At the present time, more than 80% of the reported data come from studies which either follow the segment 2 protocol (Fig. 1) or are traditional studies published in the teratology literature. This condition of the database is a natural consequence of the ongoing evolution and development of testing protocols in reproductive and developmental toxicology because assessment for reproductive (segment 1) and postnatal (segment 3) effects was given appropriate prominence much later (*11*). This paper designates reports that deal with data generated in a manner analagous to the segment 1 protocol as an evaluation of the reproductive toxicity of the chemical or mixture under question. Those reports that analyze the effects of maternal exposure during pregnancy, whether similar to the segment 2 protocol of from the literature using traditional methods of experimental teratology, are

designated as an evaluation of developmental toxicity. Finally, studies that have used some aspect of either the segment 1 or segment 3 paradigm are included here as an analysis of postnatal toxicity. In the ensuing text, an effort has been made to include the species used, the dosages used, the route of administration and, when the data permit, the designation of a dosage as the NOAEL (no-observed-adverse-effect-level).

Reproductive Toxicity

The only complex stream related to gasoline that has been tested for reproductive effects is high flash aromatic naphtha [(*20*) inhalation study in the rat, 100–150 ppm; NOAEL 100 ppm]. In this study, the only effect seen was a decrease in the weight of male pups subsequent to exposure by inhalation. This effect was seen at the two highest doses used, and the data allowed the establishment of a NOAEL at 100 ppm. No studies were available to evaluate either gasoline or its other major components. Most of the studies that were available for review did not conform to the criteria described above for a definitive segment 1 type of analysis, but are included for the sake of completeness. Exposure of male rats to ethanol (*21*) produced two effects: decreased levels of serum testosterone and histological changes in testicular morphology. Exposure of female mice, on the other hand, was without effect (*22*). When male animals of several species were exposed to benzene, the major effect observed was testicular toxicity (*23,24*). These results are summarized in Table 1. Results of studies using formaldehyde, xylene, 1,3-butadiene, and methyl tertiary butyl ether are shown in Table 2. Formaldehyde and *o*-xylene produced clear testicular toxicity (*25–27*). Mixed xylenes, administered by the inhalation route, affected mating indexes at the high dose (500 ppm), resulting in a NOAEL of 250 ppm (*28*). When male mice were exposed to 1,3-butadiene, an increase in abnormal sperm was seen as the definitive toxic effect, with a NOAEL of 200 ppm (*29*). A comprehensive investigation of methyl tertiary butyl ether, on the other hand, showed no effects on a variety of reproductive end points (*30*).

Developmental Toxicity

The ability of both naturally occurring chemicals and xenobiotics to interfere with embryonic developmental in several species and to produce abnormalities has been documented over the past 170 years (*31*). Additional observations have led to the development of a new branch of embryology which, coincident with the realization that mammalian embryos were also susceptible to the adverse effects of chemicals, become known as experimental teratology (*32*). With the delineation of the elements of the thalidomide syndrome in 1962, the discipline took on the additional status of an applied science and, since that time, has effectively used the concepts and techniques of pharmacology, toxicology, biochemistry and physiology to become an entirely new and meaningful discipline known as developmental toxicology (*6*). In this review, emphasis has been placed on the definition and guidelines that have been developed by the U.S. Environmental Protection Agency (*33*). At the present time, the manifestation of developmental toxicity after maternal exposure using the segment 2 protocol (Fig. 1) involves four interdependent elements: death of the developing organism, structural abnormalities, altered intrauterine growth, and prenatal or postnatal functional deficiency.

Gasoline and and related streams have been evaluated for their potential to produce developmental toxicity using inhalation exposure as the route of administration (Table 3). Gasoline was not a clear developmental toxicant, although skeletal variations were observed at the highest dose used, 1600 ppm (*34*). Naphtha was without effect in rats (*20,35*), but produced fetal mortality in mice, reduced fetal weight, delayed ossification, and increased the incidence of cleft palate at 1500 ppm (*20*). High aromatic solvent was also developmentally toxic at the highest dose used, producing an increase in intrauterine death (*36*). Neither rubber-solvent (*37*) nor Stoddard solvent (*38*) produced any evidence of developmental toxicity.

Of the 14 chemicals and mixtures evaluated in this review, ethanol is the only one that is a clear and unequivocal developmental toxicant in humans. The intake of eth-

Table 1. Reproductive toxicity: ethanol and benzene.

Chemical	Species	Sex	Doses	Route	Effect	Reference
Ethanol	Rat	M	6–10% ethanol	Liquid diet	Yes	(*21*)
	Mouse	F	15–35% calories	Liquid diet	No	(*22*)
Benzene	Rat	M	1–7 mL/kg	Gavage	Yes	(*23*)
	Rat	M	80, 88 ppm	Inhalation	Yes	(*24*)
	Guinea pig	M	80, 88 ppm	Inhalation	Yes	(*24*)
	Rabbit	M	80, 88 ppm	Inhalation	Yes	(*24*)

Table 2. Reproductive toxicity: formaldehyde, xylene, 1,3-butadiene, and methyl tertiary butyl ether.

Chemical	Species	Sex	Doses	Route	Effect	Reference
Formaldehyde	Rat	M	8, 16 mg/kg/day	IP	Yes	(*25*)
	Rat	M	100, 200 mg/kg	Gavage	Yes	(*26*)
o-Xylene	Rat	M	0.5, 1.0 mg/kg	IP	Yes	(*27*)
Xylene	Rat	M/F	60, 250, 500 ppm	Inhalation	Yes	(*28*)
1,3-Butadiene	Mouse	M	200–5000 ppm	Inhalation	Yes	(*29*)
Methyl tertiary butyl ether	Rat	M/F	290–2860 ppm	Inhalation	No	(*30*)

Table 3. Developmental toxicity: gasoline and related streams.

Chemical	Species	NOAEL, ppm		Developmental toxicant	Reference
		Maternal	Fetal		
Gasoline	Rat	1600	1600	No	(*34*)
High-flash naphtha	Rat	400	400	No	(*20,35*)
	Mouse	100	100	Yes	(*20*)
High aromatic solvent	Rat	100	100	Yes	(*36*)
Rubber solvent	Rat	1600	1600	No	(*37*)
Stoddard solvent	Rat	400	400	No	(*38*)

anol by the oral route has been shown to result in the birth of children that display manifestations of fetal alcohol syndrome (FAS) (*9*). These observations have led to an extensive literature on the effects of ethanol in pregnant animals. In rats, the effects observed are clearly related to the route of administration used. When exposure was by inhalation, no adverse effects were seen, and the maternal NOAEL (for narcosis) was 16,000 ppm, while the fetal NOAEL was 20,000 ppm (*39*). If, however, ethanol was administered by gavage, limb defects were observed at the doses used (*40*). No effects were seen if ethanol was administered in the drinking water (*41*). Mice have become the species of choice in efforts to develop animal models of FAS, and several studies have shown that ethanol is a clear developmental toxicant in this species (Table 4). Effects are produced in the form of clear structural malformations, and these effects are independent of the route of administration, whether oral (*42*), IP injection (*42-46*), or in a liquid diet (*22,47-49*). It is only when ethanol is administered in the drinking water that developmental toxicity is manifest primarily as reduced fetal weight (*41*). At the present time, no inhalation studies have been reported for this species. When pregnant rats are exposed to methanol by the inhalation route, however, developmental toxicity is produced and is manifest as a decrease in fetal body weight and the occurrence of structural abnormalities at high doses (*39*). In this study, the maternal NOAEL was 10,000 ppm, and the fetal NOAEL was 5000 ppm (*39*).

Toluene, a developmental toxicant in experimental animals (rats, rabbits, mice), produces its effects independently of the route of administration, although inhalation studies are predominant (*50*). A NOAEL for maternal and fetal effects has not been established. Inhalation studies have shown that benzene is also a developmental toxicant in rats (*51–55*) and mice (*56*) but not in rabbits (*54*). A NOAEL has not been established.

Table 4. Developmental toxicity of ethanol in the mouse.

Route of administration	Doses	Reference
Oral, IP	2.9, 5.8 g/kg	(*42*)
IP	0.05 mg/kg × 2	(*43*)
IP	0.03 mL/g	(*44*)
IP	0.03 mL/g	(*45*
IP	2.9–5.8 g/kg	(*46*)
Liquid diet	15–35% calories	(*22*)
Liquid diet	5.4 g/kg	(*47*)
Liquid diet	17–30% calories	(*48*)
Liquid diet	25% calories	(*49*)
Drinking water	15% v/v	(*41*)

IP, intraperitoneal

In an inhalation study that was performed for the National Toxicology Program, butadiene was shown to have no developmental toxicity with a calculated NOAEL for maternal effects of 200 ppm (*57*). For fetal effects, the NOAEL was 1000 ppm (*57*). Effects on fetal weight were reported in an earlier study (*58*). Butadiene was a clear developmental toxicant in mice, producing a reduction in mean fetal weight (*59*). These studies have been the subject of a recent, intensive review (*60*).

The ability of formaldehyde to be a developmental toxicant is directly dependent on both the route of administration and the dose. When formaldehyde was administered by gavage, it produced a high level of maternal lethality, with no statistically significant effects on any measure of developmental toxicity (*61*). Formaldehyde had no effect when applied dermally (*62*) or in the diet (*63*). When inhalation exposure was used, low doses (0–10 ppm) were without effect (*64*), although higher doses (0–20 ppm) did have an effect on mean fetal weight (*65*). This latter study provides a maternal NOAEL of 20 ppm and a fetal NOAEL of 10 ppm.

The ability of xylenes to be developmental toxicants is also dependent on the route of administration. When administered by gavage (0–4.8 mL/kg/day), xylenes are maternally toxic, producing death in the high dose range along with a spectrum of developmental effects, including an increase in fetal death, an increase in the incidence of cleft palate, and a measurable decrease in fetal weight (*66*). However, if the xylenes are administered by inhalation during pregnancy, no effects are seen (*55,67*), although the pure isomers are developmentally toxic, producing a reduction in fetal weight (*68*).

An analysis of the effects of methyl tertiary butyl ether after inhalation exposure revealed that it is a developmental toxicant in the mouse (*69,70*), with a fetal NOAEL of 1000 ppm. No effects were seen, however, when rats (*69*) or rabbits (*71*) were studied, and the fetal NOAELs are 2500 ppm and 8000 ppm, respectively (the highest doses studied). A similar trend is seen when hexane is studied for its potential as a developmental toxicant. Commercial hexane is effective in mice only at the highest dose used (9000 ppm; *72*) but is without effect in rats at the same level of exposure (*73*). Pure *n*-hexane is also without effect (*74,75*).

Two studies assessed potential dose–resonse characteristics and suggested that one of the most sensitive measures of developmental toxicity is an observable reduction in mean fetal weight (*76,77*). This single developmental effect has been reported for 10 of the 14 gasoline-related materials that were reviewed (Table 5). The clear prevalence of this response, often in the absence of any other

Table 5. Chemicals that produce reductions in fetal weight.

Chemical	Reference
High-flash naphtha	(*20*)
Ethanol	(*40*)
Methanol	(*39*)
Toluene	(*50*)
Benzene	(*51,54,55*)
Formaldehyde	(*65*)
Xylene	(*68*)
1,3-Butadiene	(*60*)
Methyl tertiary butyl ether	(*70*)
Hexane	(*75*)

clear manifestation of developmental toxicity, lends independent support to the suggestion that there may be a relationship between this sensitive parameter of developmental toxicity and the potential ability of a chemical to produce birth defects at a higher dose level. It also validates the current requirement that, for a statistically valid developmental toxicity study, a minimum of three dose levels is essential to clearly define underlying dose–response relationships.

Postnatal Toxicity

Of the 14 chemicals and mixtures that were evaluated, there was sufficient data for analysis for only 6 of them for evidence of postnatal toxicity. A summary of the data obtained in these studies is contained in Table 6.

The analysis of the reproductive, developmental, and postnatal effects of high-flash aromatic naphtha was reported in a complete multigenerational study in which the basic protocol for the segment 1 study was extended to include an evaluation of offspring through the F_3 generation with continual inhalation exposure (*20*). Effects were seen in all generations, but became progressively severe, particularly at the high dose (1500 ppm) where, in the F_3 group, most of the males and females from the F2 generation died during the first week of exposure. Behavioral effects seen in this generation included ataxia and reduced motor activity.

Most of the other studies designed to examine postnatal effects used some variation of the segment 3 protocol (Fig. 1) in which only the pregnant dams were exposed. With ethanol, treatment of pregnant rats by gavage produced decreased litter weight in both dose groups studied and a concomitant dose-dependent increase in postnatal mortality in the exposed pups. Impaired motor activity was also observed (*78*). When ethanol was administered in a liquid diet, newborn animals had a low birth weight (*79*). In mice, prenatal ethanol exposure is associated with the occurrence of a high level of unilateral hydronephrosis on postnatal day 21, an effect not observed on postnatal day 35 (*47*). In addition, effects on the postnatal weight profile have also been reported (*80*).

In a well-designed and comprehensive study, methanol has been shown to be a clear behavioral teratogen in rats, an effect seen in the absence of any other manifestation of perinatal toxicity (*81*). Toluene was a postnatal toxicant in rats, producing effects on the weight of exposed pups (*82*) and on the postnatal development of the hippocampus (*83*). No effects were seen in mice (*84*). When xylene was studied in a full segment 1 study (*28*), effects were seen on the weights of exposed pups, but only at the highest doses tested. Methyl tertiary butyl ether was without effect (*30*).

Recommendations

This review was compiled with the objective of presenting a comprehensive database on the reproductive and developmental toxicity of 14 select chemicals and mixtures. A major deficit in that database is the relatively incomplete nature of several studies, both with respect to the number of species that have been studied in the three segment tests and with respect to dose levels. This is most apparent in the reproduction (segment 1) and postnatal (segment 3) studies. Additionally, attention should be paid to the route of administration in any attempt to standardize, expand upon, and validate the data reported here. This database provides a useful starting point, both for further studies that use the standard protocols and their specific variations and for future mechanistic studies to delineate those targets that are affected and that lead to measurable toxicity.

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Table 6. Postnatal toxicity of selected chemicals.

Chemical	Species	Route	Sex	Doses	Effects[a]		Reference
					Behavioral	Other	
High-flash naphtha	Rat	Inhalation	M/F	100–1500 ppm	Yes	Yes	(*20*)
Ethanol	Rat	Gavage	F	4,6 g/L	Yes	Yes	(*78*)
	Rat	Liquid diet	F	50 g/L	–	Yes	(*79*)
	Mouse	Liquid diet	F	30% calories	–	Yes	(*47*)
	Mouse	Liquid diet	F	25% calories	–	Yes	(*80*)
Methanol	Rat	Drinking water	F	2% v/v	Yes	–	(*81*)
Toluene	Rat	SC	F	1.25 g/kg	–	Yes	(*82*)
	Rat	Inhalation	F	100, 500 ppm	–	Yes	(*83*)
	Mouse	Inhalation	F	200, 400 ppm	–	No	(*84*)
Xylene	Rat	Inhalation	F	60–500 ppm	–	Yes	(*28*)
Methyl tertiary butyl ether	Rat	Inhalation	F	290–2860 ppm	–	No	(*30*)

[a]A dash indicates not evaluated.

REFERENCES

1. Korach, K. S., and Quarmby, V. E. Morphological, physiological, and biochemical aspects of female reproduction. In: Reproductive Toxicology (R. E. Dixon, Ed.), Raven Press, New York, 1985, pp. 47–68.
2. Desjardins, C. Morphological, physiological, and biochemical aspects of male reproduction. In: Reproductive Toxicology (R. E. Dixon, Ed.), Raven Press, New York, 1985, pp. 131–146.
3. Yanagamachi, R. Mammalian fertilization. In: The Physiology of Reproduction, Vol. 1 (E. Knobil and J. Neill, Eds.), Raven Press, New York, 1988, pp. 135–185.
4. Weitlauf, H. M. Biology of implantation. In: The Physiology of Reproduction, Vol. 1 (E. Knobil and J. Neill, Eds.), Raven Press, New York, 1988, pp. 231–262.
5. Hoar, R. M., and Monie, I. W. Comparative development of specific organ systems. In: Developmental Toxicology (C. A. Kimmel and J. Buelke-Sam, Eds.), Raven Press, New York, 1981, pp. 13–33.
6. Skalko, R. G. Biochemical mechanismsindevelopmental toxicology. In: Developmental Toxicology (C. A. Kimmel and J. Buelke-Sam, Eds.), Raven Press, New York, 1981, pp. 1–11.
7. Skalko, R. G. Pharmacological concepts and developmental toxicology. Ann. N.Y. Acad. Sci. 562: 21–30 (1989).
8. Hood, R. D., Ed. Developmental Toxicology: Risk Assessment and the Future. Van Nostrand Reinhold, New York, 1989.
9. Streissguth, A. P., Landesman-Dwyer, S., Martin, J. C., and Smith, D. W. Teratogenic effects of alcohol in humans and laboratory animals. Science 209: 353–361 (1980).
10. Lalonde, M., Ed. The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity. Health and Welfare Canada, Ottawa, 1973.
11. Palmer, A. K. Regulatory requirements for reproductive toxicology: theory and practice. In: Developmental Toxicology (C. A. Kimmel and J. Buelke-Sam, Eds.), Raven Press, New York, 1981, pp. 259–287.
12. Khera, K. S., Grice, H. C., and Clegg, D. J., Eds. Current Issues in Toxicology: Interpretation and Extrapolation of Reproductive Data to Establish Human Safety Standards. Springer-Verlag, New York, 1989.
13. Heinrichs, W. L. Current laboratory approaches for assessing female reproductive toxicity. In: Reproductive Toxicology (R. E. Dixon, Ed.), Raven Press, New York, 1985, pp. 95–108.
14. Wilson, J. G. Environment and Birth Defects. Academic Press, New York, 1973.
15. Dixon, R. D. Toxic responses of the reproductive system. In: Casarett and Doull's Toxicology. The Basic Science of Poisons, 3rd ed. (C. D. Klaassen, M. O. Amdur, and J. Doull, Eds.), Macmillan, New York, 1986, pp. 432–477.
16. Nau, H. Species differences in pharmacokinetics and drug teratogenesis. Environ. Health Perspect. 70: 113–129 (1986).
17. Barlow, S. M., and Sullivan, F. M. Reproductive Hazards of Industrial Chemicals. Academic Press, London, 1982.
18. Schardein, J. L. Chemically Induced Birth Defects. Marcel Dekker, New York, 1985.
19. Shepard, T. H. Catalog of Teratogenic Agents, 6th ed. Johns Hopkins University Press, Baltimore, MD, 1989.
20. McKee, R. H., Wong, Z. A., Schmitt, S., Beatty, P., Swanson, M., Schreiner, C. A., and Schardein, J. L. The reproductive and developmental toxicity of high flash aromatic naphtha. Toxicol. Ind. Health 6: 441–460 (1990).
21. Klassen, R. W., and Persaud, T. V. N. Influence of alcohol on the reproductive system of the male rat. Int. J. Fertil. 23: 176–184 (1978).
22. Chernoff, G. F. The fetal alcohol syndrome in mice: an animal model. Teratology 15: 223–229.
23. Spano, M., Pacchierotti, F., Vecelli, R., Amendola, R., and Bartoleschi, C. Cytotoxic effects of benzene on mouse germ cells determined by flow cytometry. J. Toxicol. Environ. Health 26: 361–372 (1989).
24. Wolf, M. A., Rowe, V. K., McCollister, D. D., Hollingsworth, R. L., and Oyen, F. Toxicological studies of certain alkylated benzenes and benzene. Arch. Ind. Health 14: 387–398 (1956).
25. Shah, B. M., Vachhrajani, K. D., Chinoy, M. J., and Choudhuri, A. R. Formaldehyde induced structural and functional changes in the testes of the rat. J. Reprod. Biol. Comp. Endocrinol. 7: 42–52 (1987).
26. Cassidy, S. L., Dix, K. M., and Jenkins, T. Evaluation of a sperm head counting technique using rats exposed to dimethoxyethyl phthalate (DMAP), glycerol-α-monochlorohydrin (GMCH), epichlorhydrin (ECH), formaldehyde (FA) or methyl methane suphonate (MMS). Arch. Toxicol. 53: 71–78 (1983).
27. Washington, W. J., Murthy, R. C., Doyle, A., Eugene, K., Brown, D., and Bradley, I. Induction of morphological abnormal sperm in rats exposed to o-xylene. Arch. Androl. 11: 233–237 (1983).
28. Schroeder, R. E., and Daly, I. W. Parental and Foetal Reproduction Inhalation Toxicity in Rats with Mixed Xylenes. Final Report, Project 80-2520, Biodynamics, Inc., 1983.
29. Hackett, P. L., Brown, M. G., Clark, K. L., Evanoff, J. J., Rowe, S. E., McClanahan, B. J., Buchsbom, R. L., Decker, J. R., Rommereim, R. L., and Westerberg, R. B. Sperm Head Morphology Study in B6C3F1 Mice following Inhalation Exposure to 1,3-Butadiene. Final Report, NIH-Y01-NIEHS-ES-70153, 1988.
30. Biles, R. W., Schroeder, R. W., and Holdsworth, C. E. Methyl tertiary butyl ether inhalation in rats: a single generation reproduction study. Toxicol. Ind. Health 3: 519–534 (1987).
31. Corner, G. W. Congenital malformations: the problem and the task. In: First International Conference on Congenital Malformations (M. Fishbein, Ed.), J. B. Lippincott, Philadelphia, 1961, pp. 7–17.
32. Persaud, T. V. N., Chudley, A. E., and Skalko, R. G., Eds. Basic Concepts in Teratology. Alan R. Liss, New York, 1985.
33. U.S. Environmental Protection Agency. Proposed guidelines for the health assessment of suspect developmental toxicants. Fed. Reg. 49 (227): 46324–46331 (1984).
34. Beliles, R. P. Teratology Study in Rats: Unleaded Gasoline. Litton Bionetics Project No. 20698-6, Litton Bionetics, Inc., Bethesda, MD, 1978.
35. Beliles, A. P., and Mecler, F. J. Inhalation teratology of jet fuel A, fuel oil and petroleum naphtha in rats. In: Toxicology of Petroleum Hydrocarbons (H. N. McFarland, C. E. Holdsworth, J. A. MacGregor, R. W. Call, and M. L. Kane, Eds.), 1982, pp. 223–238.
36. Beliles, R. P., and Weir, R. J. Teratology Study in Rats: High Aromatic Solvent. Final Report, Project No. 20689-7, Litton Bionetics, Inc., Bethesda, MD, 1979.
37. Beliles, R. P., and Weir, R. J. Teratology Study in Rats: Rubber Solvent. Final Report, Project No. 26-60015, Litton Bionetics, Inc., Bethesda, MD, 1978.
38. Beliles, R. P., and Weir, R. J. Teratology Study in Rats: Stoddard Solvent. Project No. 20698-2, Litton Bionetics, Inc., Bethesda, MD, 1978.
39. Nelson, B. K., Brightwell, W. S., MacKenzie, D. R., Khan, A., Burg, J. R., Weigel, W. W., and Goad, P. T. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam. Appl. Toxicol. 5: 727–736 (1985).
40. West, J. R., Black, A. C., Jr., Reimann, P. C., and Alkana, R. L. Polydactyly and polysyndactyly induced by prenatal exposure to ethanol. Teratology 24: 13–18 (1981).
41. Schwetz, B. A., Smith, F. A., and Staples, R. E. Teratogenic potential of ethanol in mice, rats and rabbits. Teratology 18: 385–392 (1978).
42. Webster, W. S., Walsh, D. A., McEwen, S. E., and Lipson, A. H. Some teratogenic properties of ethanol and acetaldehyde in C57 Bl/6J mice: implications for the study of the fetal alcohol syndrome. Teratology 27: 231–243 (1983).
43. Sulik, K. K., and Johnston, M. C. Sequence of developmental alternatives following acute ethanol exposure in mice: craniofacial features of the fetal alcohol syndrome. Am. J. Anat. 166: 257–259 (1983).
44. Bannigan, J., and Burke, P. Ethanol teratogenicity in mice: a light microscope study. Teratology 26: 247–254 (1982).
45. Kronick, J. B. Teratogenic effects of ethyl alcohol administered to pregnant mice. Am. J. Obstet. Gynecol. 124: 676–680 (1976).
46. Webster, W. S., Walsh, D. A., Lipson, A. H., and McEwen, S. E. Teratogenesis after acute alcohol exposure in inbred and outbred mice. Neurobehav. Toxicol. 2: 227–234 (1980).
47. Boggan, W. O., and Randall, C. M. Renal anomalies in mice prenatally exposed to ethanol. Chem. Pathol. Pharmacol. 23: 127–142 (1979).
48. Randall, C. M., and Taylor, W. J. Prenatal ethanol exposure in mice: teratogenic effects. Teratology 19: 305–312 (1979).
49. Randall, C. M., Taylor, W. J., and Walker, D. W. Ethanol-induced malformations in mice. Alcoholism 1: 219–224 (1977).
50. Donald, J. M., Hooper, K., and Hopenhayn-Rich, C. Reproductive and developmental toxicity of toluene: a review. Environ. Health Perspect. 94: 237–244 (1991).

51. Coate, W. B. , Hoberman, A. M., and Durloo, R. S. Inhalation teratology study of benzene in rats. In: Advances in Modern Environmental Toxicology, Vol. 6 (H. N. MacFarland, C. E. Holdsworth, J. A. MacGregor, R. W. Call, and M. L. Lane, Eds.), Princeton Scientific Publishers, Princeton, NJ, 1984, pp. 187–198.
52. Kuna, R. A., and Kapp, R. W., Jr. The embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol. Appl. Pharmacol. 57: 1–7 (1981).
53. Green, J. D., Leong, B. K. J., and Lankin, S. Inhaled benzene fetotoxicity in rats. Toxicol. Appl. Pharmacol. 46: 9–18 (1978).
54. Tatrai, E., Ungvary, G., Hudak, A., Rocids, K., Lorincz, M., and Barcza, G. Concentration dependence of the embryotoxic effects of benzene inhalation in CFY rats. J. Hyg. Epidemiol. Microbiol. Immunol. 24: 363–371 (1980).
55. Hudak, A., and Ungvary, G. Embryotoxic effects of benzene and its methyl derivatives: Toluene and xylene. Toxicology 11: 55–63 (1978).
56. Murray, F. J., John, J. A., Rampy, L. W., Kuna, R. A., and Schwetz, B. A. Embryotoxicity of inhaled benzene in mice and rabbits. Am. Ind. Hyg. Assoc. J. 40: 993–998 (1979).
57. Hackett, P. L., Sikov, M. R., Mast, T. G., Brown, M. G., Buchsbom, R. L., Clark, M. L., Decker, J. R., Evanoff, J. J., Romereim, R. L., Rowe, S. E., and Westerberg, R. B. Inhalation Developmental Toxicity Studies of 1,3-Butadiene in the Rat. Final Report, NIH-ES-40131, NIEHS 88-004186, 1987.
58. Irvine, L. F. H. 1,3-Butadiene: Inhalation Teratogenicity Study in the Rat. Final Report 2788-522/3. Hazelton Laboratories, Madison, WI, 1981.
59. Hackett, P. L., Sikov, M. R., Mast, T. J., Brown, M. G., Buchsbom, R. L., Decker, J. R., Rommerein, R. L., and Westerberg, R. B. Inhalation Developmental Toxicity Studies: Teratology Study of 1,3-Butadiene in Mice. Report No. PNL-6412-UC-48, U.S. Department of Energy, Washington, DC, 1987.
60. Morrissey, R. E., Schwetz, B. A., Hackett, P. L., Sikov, M. R., Hardin, B. D., McClanahan, B. J., Decker, J. R., and Mast, T. J. Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. Environ. Health Perspect. 86: 79–94 (1990).
61. Marks, T. A., Worthy, W. C., and Staples, R. E. Influence of formaldehyde and sonacide (potentiated acid glutaraldehyde) on embryo and fetal development in mice. Teratology 22: 51–58 (1980).
62. Overman, D. O. Absence of embryotoxic effects of formaldehyde after percutaneous exposure in hamsters. Toxicol. Lett. 24: 107–110 (1985).
63. Hurni, H., and Ohder, H. Reproduction study with formaldehyde and hexamethylenetetramine in beagle dogs. Food Cosmet. Toxicol. 11: 459–462 (1973).
64. Martin, W. J. A teratology study of inhaled formaldehyde in the rat. Reprod. Toxicol. 4: 237–239 (1990).
65. Saillenfait, A. M., Bonnet, P., and De Ceaurriz, J. The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem. Toxicol. 27: 545–548 (1989).
66. Marks, T. A., Ledoux, T. A., and Moore, J. A. Teratogenicity of a commercial xylene mixture in the mouse. J. Toxicol. Environ. Health 2: 97–105 (1982).
67. Beliles, R. P., and Weir, R. J. Teratology Study in Rats: Xylene. Project No. 20698-5, Litton Bionetics, Inc., Bethesda, MD, 1978.
68. Ungvary, G., Tatrai, E., Hudak, A., Barcza, G., and Lorincz, M. Studies on the embryotoxicity of otho-, meta-, and para-xylene. Toxicology 18: 61–74 (1980).
69. Conaway, C. C., Schroeder, R. E., and Snyder, N. K. Teratology evaluation of methyl tertiary butyl ether in rats and mice. J. Toxicol. Environ. Health 16: 797–809 (1985).
70. Tyl, R. W., Neeper-Bradley, T. L., and Frank, F. R. Developmental Toxicity Study of Inhaled Methyl Tertiary Butyl Ether in CD1 Mice. Report No. 52-526, Bushy Run Research Center, 1989.
71. Tyl, R. W., and Frank, F. R. Developmental Toxicity of Inhaled Methyl Tertiary Butyl Ether in New Zealand White Rabbits. Report No. 51-628, Bushy Run Research Center, 1989.
72. Neeper-Bradley, T. L. Developmental Toxicity Study of Commercial Hexane Vapor in CD1 mice. Report No. 52-604, Bushy Run Research Center, 1989.
73. Neeper-Bradley, T. L., and Frank, F. R. Developmental Toxicity of Commercial Hexane in CD (Sprague-Dawley) Rats. Report No. 52-605, Bushy Run Research Center, 1989.
74. Beliles, R. P., and Weir, R. J. Teratology study in rats: n-hexane. Final Report, Project No. 20698-9, Litton Bionetics, Inc., Bethesda, MD, 1979.
75. Mast, T. J. Inhalation Developmental Toxicology Studies: Teratology Study of n-Hexane in Rats. Report 99352, Pacific Northwest Laboratory, Richland, WA, 1987.
76. Ryan, L. M., Catalano, P. J., Kimmel, C. A., and Kimmel, G. L. Relationship between fetal weight and malformation in developmental toxicity studies. Teratology 44: 215–223 (1991).
77. Knudsen, T. B., Winters, R. S., Otey, S. L., Blackburn, M. R., Airhart, M. J., Church, J. K., and Skalko, R. G. Effects of (R)-deoxycoformycin (pentostatin) on intrauterine nucleoside catabolism and embryo viability in the pregnant mouse. Teratology 45: 91–103 (1992).
78. Abel, E. L., and Dintcheff, B. A. Effects of prenatal alcohol exposure on growth and development in rats. J. Pharmacol. Exp. Therap. 207: 916–921 (1978).
79. Weiner, S. G., Shoemaker, W. J., Koda, L. Y., and Bloom, F. E. Interaction of ethanol and nutrition during gestation: influence on maternal and offspring development in the rat. J. Pharmacol. Exp. Therap. 216: 572–579 (1981).
80. Middaugh, L. D., Randall, C. L., and Favaro, J. P. Prenatal ethanol exposure in C57 mice: effects on pregnancy and development. Neurotoxicol. Teratol. 10: 175–180 (1988).
81. Infurna, R., and Weiss, B. Neonatal behavioral toxicity in rats following prenatal exposure to methanol. Teratology 33: 259–265 (1986).
82. da Silva, V. A., Malheiros, L. R., Paumgartten, F. J. R., De Matos Sa Rego, M., Riul, T. R., and Golvattei, M. A. R. Developmental toxicity of in utero exposure to toluene in malnourished and well nourished rats. Toxicology 64: 155–168 (1990).
83. Slomianka, L., Edelfors, S., Ravin-Jonsen, A., Rungby, J., Danscher, G., and West, M. J. The effects of low-level toluene exposure on the developing hippocampal region of the rat: histological evidence and volumetric findings. Toxicology 62: 189–202 (1990).
84. Courtney, K. D., Andrews, J. E., Springer, J., Menache, M., Williams, T., Dalley, L., and Graham, J. A. A perinatal study of toluene in CD-1 mice. Fundam. Appl. Toxicol. 6: 145–154 (1986).